

What is claimed is:

- 1 1. (Original) An isolated hyperactive reverse transcriptase comprising one or more point  
2 mutations in the processivity domain and one or more point mutations in the nucleotide selection  
3 domain.
- 1 2. (Currently Amended) The reverse transcriptase of claim 1, wherein the reverse  
2 transcriptase is selected from the group consisting of AMV, M-MLV, HTLV-1, BLV, RSV,  
3 HFV, R2-Bombyx mori, and HIV reverse transcriptase.
- 1 3. (Original) The reverse transcriptase of claim 1, wherein the reverse transcriptase is  
2 encoded by a modified nucleotide sequence that encodes a modified amino acid sequence  
3 modified in the processivity domain corresponding to amino acids 497 to 671 of M-MLV reverse  
4 transcriptase.
- 1 4. (Original) The reverse transcriptase of claim 1, wherein the reverse transcriptase is  
2 encoded by a modified sequence that encodes a modified amino acid sequence modified in the  
3 nucleotide selection domain corresponding to amino acids 153 to 158 of M-MLV reverse  
4 transcriptase.
- 1 5. (Original) The reverse transcriptase of claim 1, wherein the reverse transcriptase may be  
2 used in the preparation of full-length cDNA.
- 1 6. (Original) The reverse transcriptase of claim 1, wherein the reverse transcriptase  
2 comprises reverse transcriptase produced recombinantly.
- 1 7. (Original) The reverse transcriptase of claim 1, wherein the reverse transcriptase is  
2 purified.
- 1 8. (Original) The reverse transcriptase of claim 1, wherein the reverse transcriptase is  
2 purified and is greater than 90% pure.

1 9. (Currently Amended) The reverse transcriptase of claim 1, wherein the mutation in the  
2 processivity domain comprises one or more of mutations in the following residues in MMLV-  
3 RT: H638, ~~Y586~~, ~~D653~~, ~~D524~~, ~~D524~~ and ~~E562~~.

1 10. (Cancelled)

1 11. (Currently Amended) The reverse transcriptase of claim 1, wherein the mutation in the  
2 nucleotide selection domain comprises one or more mutations in the following residues in  
3 MMLV-RT: F155, ~~D153~~, ~~A154~~, ~~F155~~, ~~F156~~, ~~C157~~, or ~~L158~~.

1 12. (Currently Amended) The reverse transcriptase of claim 1, wherein the mutation in the  
2 processivity domain comprises one or more of the following mutations corresponding to the  
3 amino acids in MMLV-RT: H638G, ~~Y586A~~, ~~D653N~~, ~~D524N~~, ~~D524E~~ and ~~E562D~~ and the  
4 mutation in the nucleotide selection domain comprises F155Y.

1 13. (Original) The reverse transcriptase of claim 1, wherein the reverse transcriptase  
2 produces a yield of greater than about 1 ug of an aRNA from 100 ng of template RNA in a single  
3 amplification reaction.

1 14. (Original) The reverse transcriptase of claim 1, wherein the reverse transcriptase  
2 produces a yield of greater than about 5 ug of an aRNA from 100 ng of template RNA in a single  
3 amplification reaction.

1 15. (Original) The reverse transcriptase of claim 1, wherein the reverse transcriptase  
2 produces a yield of greater than about 7 ug of an aRNA from 100 ng of template RNA in a single  
3 amplification reaction.

1 16. (Original) The reverse transcriptase of claim 1, wherein the reverse transcriptase  
2 produces a yield of greater than about 10 ug of an aRNA from 100 ng of template RNA in a  
3 single amplification reaction.

1 17. (Original) The reverse transcriptase of claim 1, wherein the reverse transcriptase  
2 produces a yield of greater than about 15 ug of an aRNA from 100 ng of template RNA in a  
3 single amplification reaction.

1 18. (Original) The reverse transcriptase of claim 1, wherein the reverse transcriptase  
2 produces a yield of greater than about 25 ug of an aRNA from 100 ng of template RNA in a  
3 single amplification reaction.

1 19. (Original) The reverse transcriptase of claim 1, wherein the reverse transcriptase  
2 produces a yield of greater than about 1 ug of an aRNA from 10 pg of template RNA after a two-  
3 round amplification reaction.

1 20. (Original) The reverse transcriptase of claim 1, wherein the reverse transcriptase  
2 produces a yield of greater than about 2 ug of an aRNA from 10 pg of template RNA after a two-  
3 round amplification reaction.

1 21. (Original) The reverse transcriptase of claim 1, wherein the reverse transcriptase  
2 produces a yield of greater than about 5 ug of an aRNA from 10 pg of template RNA after a two-  
3 round amplification reaction.

1 22. (Original) The reverse transcriptase of claim 1, wherein the reverse transcriptase  
2 produces a yield of greater than about 10 ug of an aRNA from 10 pg of template RNA after a  
3 two-round amplification reaction.

1 23. (Original) The reverse transcriptase of claim 1, wherein the reverse transcriptase  
2 produces a cDNA greater than about 6, 9 or even 11 kilobases in a single cDNA synthesis  
3 reaction.

1 24. (Original) The reverse transcriptase of claim 1, wherein the reverse transcriptase  
2 produces a cDNA greater than about 6 to about 15 kilobases in a single cDNA synthesis  
3 reaction.

- 1 25. (Original) The reverse transcriptase of claim 1, wherein the reverse transcriptase  
2 produces a cDNA greater than about 15 kilobases in a single cDNA synthesis reaction.
- 1 26. (Original) The reverse transcriptase of claim 1, wherein the DNA polymerase activity is  
2 greater than about 200 Units per microgram.
- 1 27. (Original) The reverse transcriptase of claim 1, wherein the DNA polymerase activity is  
2 between about 0.1 and 300 Units per microgram.
- 1 28. (Original) The reverse transcriptase of claim 1, wherein the RNase H activity is between  
2 about 0.1 and about 25 percent of the wild-type RNase H activity.
- 1 29 -45. (Cancelled)
- 1 46. (Original) An isolated and purified reverse transcriptase protein comprising one or more  
2 mutations in the nucleotide selection domain.
- 1 47. (Currently amended) The reverse transcriptase of claim 46, wherein the reverse  
2 transcriptase is selected from the group consisting of AMV, M-MLV, HTLV-1, BLV, RSV,  
3 HFV, R2-Bombyx mori, and HIV reverse transcriptase.
- 1 48. (Original) The reverse transcriptase of claim 46, wherein the reverse transcriptase further  
2 comprises a modified nucleotide sequence that encodes a modified amino acid sequence in the  
3 processivity domain corresponding to amino acids 497 to 671 of M-MLV reverse transcriptase.
- 1 49. (Original) The reverse transcriptase of claim 46, further comprising one point mutation  
2 in the nucleotide selection domain corresponding to amino acids 153 to 158 of MMLV reverse  
3 transcriptase.
- 1 50. (Original) The reverse transcriptase of claim 46, wherein the reverse transcriptase may  
2 be used in the preparation of full-length cDNA.

1 51. (Currently amended) The reverse transcriptase of claim 46, wherein the mutation in the  
2 processivity domain comprises one or more of the following mutations corresponding to the  
3 amino acids in MMLV-RT: H638G, Y586A, D653N, D524N, D524E and E562D.

1 52. (Original) The reverse transcriptase of claim 46, wherein the reverse transcriptase  
2 produces a yield of greater than about 1, 5, 7, 10, 12, 15, 25, 40 or 50 ug of an aRNA from 100  
3 ng of template RNA in a single amplification reaction.

1 53. (Original) The reverse transcriptase of claim 46, wherein the reverse transcriptase  
2 produces a yield of greater than about 1, 5 or 10 ug of an aRNA from 10 pg of template RNA  
3 after a double amplification reaction.

1 54. (Original) The reverse transcriptase of claim 46, wherein the reverse transcriptase  
2 produces a cDNA greater than about 6, 9 or even 11 kilobases in a single cDNA synthesis  
3 reaction.

1 55. (Original) The reverse transcriptase of claim 46, wherein the reverse transcriptase  
2 produces a cDNA greater than about 15 kilobases in a single cDNA synthesis reaction.

1 56. (Original) The reverse transcriptase of claim 46, wherein the DNA polymerase activity is  
2 greater than about 200 Units per microgram.

1 57. (Original) The reverse transcriptase of claim 46, wherein the DNA polymerase activity is  
2 between about 0.1 and 300 Units per microgram.

1 58. (Original) The reverse transcriptase of claim 46, wherein the RNase H activity is  
2 between about 0.1 and about 25 percent of the wild-type RNase H activity.

1 59. (Original) A reverse transcriptase protein comprising one or more mutations in the  
2 nucleotide selection domain and in the processivity domain.

1 60. (Original) An isolated and purified protein comprising one or more mutations in the  
2 processivity domain and one or more mutations in the nucleotide selection domain.

1 61 - 83. (Cancelled)

1 84. (Original) A hyperactive reverse transcriptase in which one or more mutations replace at  
2 least one of the amino acids of the processivity domain and the nucleotide selection domain, with  
3 an alternative naturally occurring L-amino acid, the replacement being selected from the group  
4 consisting of: (1) a substitution of any of isoleucine, valine, and leucine for any other of these  
5 amino acids; (2) a substitution of aspartic acid for glutamic acid or vice versa; (3) a substitution  
6 of glutamine for asparagine or vice versa; (4) a substitution of serine for threonine or vice versa;  
7 (5) a substitution of glycine for alanine or vice versa; (6) a substitution of alanine for valine or  
8 vice versa; (7) a substitution of methionine for any of leucine, isoleucine, or valine and vice  
9 versa; and (8) a substitution of lysine for arginine or vice versa.

1 85. (Original) The reverse transcriptase of claim 84, wherein the replacement is selected  
2 from the group consisting of: (1) a substitution of any of isoleucine, valine, or leucine for any  
3 other of these amino acids; (2) a substitution of aspartic acid for glutamic acid or vice versa; (3)  
4 a substitution of glutamine for asparagine or vice versa; and (4) a substitution of serine for  
5 threonine or vice versa and wherein the hyperactive reverse transcriptase comprises a  
6 hyperactive reverse transcriptase.

1 86. (Original) A kit for nucleic acid synthesis, comprising, in a suitable container:  
2 a hyperactive reverse transcriptase; and  
3 a reaction solution for the reverse transcriptase.

1 87. (Original) The kit of claim 86, further comprising an insert that comprises information  
2 for using the reverse transcriptase.

1 88. (Original) The kit of claim 86, wherein the reaction solution comprises a reverse  
2 transcriptase reaction buffer.

1 89. (Original) The kit of claim 86, further comprising a primer.

- 1 90. (Original) The kit of claim 86, wherein the reaction solution comprises a reverse  
2 transcriptase buffer.
- 1 91. (Original) The kit of claim 86, wherein the reaction solution comprises a PCR buffer.
- 1 92. (Original) The kit of claim 86, further comprising a mix of nucleotides.
- 1 93. (Original) The kit of claim 86, further comprising containers comprising individual  
2 nucleotides.
- 1 94. (Original) The kit of claim 86, wherein the reaction solution comprises a buffer for in  
2 vitro transcription.
- 1 95. (Original) The kit of claim 86, further comprising a template purification column.
- 1 96. (Original) The kit of claim 86, further comprising magnetic particles suitable for nucleic  
2 acid purification.
- 1 97. (Original) A kit for nucleic acid synthesis, comprising, in a suitable container:  
2 a hyperactive reverse transcriptase comprising one point mutation in the processivity domain;  
3 and  
4 a reaction solution for the reverse transcriptase.
- 1 98. (Original) A kit for nucleic acid synthesis, comprising, in a suitable container:  
2 a hyperactive reverse transcriptase comprising one point mutation in the processivity domain and  
3 one point mutation in the nucleotide selection domain; and  
4 a reaction solution for the reverse transcriptase.
- 1 99 - 101. (Cancelled)
- 1 102. (Original) A kit for RNA amplification, comprising, in a suitable container:

2 a hyperactive reverse transcriptase comprising one or more point mutations in the processivity  
3 domain and one or more point mutations in the nucleotide selection domain; an oligonucleotide  
4 comprising a transcriptional promoter region and oligo(dT) region; a DNA polymerase; and an  
5 RNA polymerase.

1 103. (Original) The kit of claim 102, further comprising an insert that comprises information  
2 for using the optimized reverse transcriptase.

1 104. (Original) The kit of claim 102, wherein the reaction solution comprises a 10X  
2 concentrated reverse transcriptase reaction buffer.

1 105. (Original) The kit of claim 102, further comprising a primer.

1 106. (Original) The kit of claim 102, wherein the reaction solution comprises a reverse  
2 transcriptase buffer.

1 107. (Original) The kit of claim 102, wherein the reaction solution comprises a DNA  
2 Polymerase buffer.

1 108. (Original) The kit of claim 102, further comprising a mix of nucleotides.

1 109. (Original) The kit of claim 102, further comprising containers comprising individual  
2 nucleotides.

1 110. (Original) The kit of claim 102, wherein the reaction solution comprises a buffer for in  
2 vitro transcription.

1 111. (Original) The kit of claim 102, further comprising a nucleic acid purification column.

1 112. (Original) The kit of claim 102, further comprising a magnetic particle or particles  
2 suitable for nucleic acid purification.

1 113 - 114. (Cancelled)

1 115. (Original) A kit for RNA amplification, comprising, in a suitable container:



2 a hyperactive reverse transcriptase comprising one or more point mutations in the processivity  
3 domain; an oligonucleotide comprising a transcriptional promoter region and oligo(dT) region; a  
4 DNA polymerase; and an RNA polymerase.

1 116. (Original) The kit of claim 115, further comprising an insert that comprises information  
2 for using the optimized reverse transcriptase.

1 117. (Original) The kit of claim 115, wherein the reaction solution comprises a 10X  
2 concentrated reverse transcriptase reaction buffer.

1 118. (Original) The kit of claim 115, further comprising a primer.

1 119. (Original) The kit of claim 115, wherein the reaction solution comprises a reverse  
2 transcriptase buffer.

1 120. (Original) The kit of claim 115, wherein the reaction solution comprises a DNA  
2 polymerase buffer.

1 121. (Original) The kit of claim 115, further comprising a mix of nucleotides.

1 122. (Original) The kit of claim 115, further comprising containers comprising individual  
2 nucleotides.

1 123. (Original) The kit of claim 115, wherein the reaction solution comprises a buffer for in  
2 vitro transcription.

1 123~~4~~. (Original) The kit of claim 115, further comprising a nucleic acid purification column.

1 125. (Original) The kit of claim 115, further comprising one or more magnetic particles  
2 suitable for nucleic acid purification.

1 126. (Cancelled)

1     127.     (Original) An RT-PCR kit comprising in one or more suitable containers: a hyperactive  
2     reverse transcriptase, two or more primers, nucleotides, a thermostable DNA polymerase and an  
3     RT-PCT buffer.

1     128.     (Original) The RT-PCR kit of claim 127, wherein the container comprising a hyperactive  
2     reverse transcriptase further comprises one or more reverse transcriptases.